Pharmacology and Mechanism of Action of Cryptenamine

By BHAGAVAN S. JANDHYALA* and JOSEPH P. BUCKLEY

Cryptenamine, an alkaloidal preparation from *Veratrum viride*, was investigated for hypotensive activity. Simultaneous bilateral denervation of the carotid sinus-body complex and bilateral vagotomy abolished the effects of cryptenamine. Adrenalectomy or pretreatment of the animals with *N,N*-di-isopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286), bretylium, reserpine, or α methyldopa abolished or markedly inhibited the hypotensive effects of cryptenamine, whereas guanethidine, which does not induce depletion of catecholamines from the adrenal medulla, failed to block the hypotensive effects of cryptenamine. Central depressor effects of cryptenamine were not abolished either in dog cross-circulation preparations or in the perfused lateral ventricle preparation of the cat. Although cryptenamine induced a depressor response in the body of the recipient following the administration of the drug into the carotid inflow to the recipient's head in the cross-circulation experiment, these responses were essentially abolished by bilateral denervation of carotid sinus-body complex. Cryptenamine potentiated epinephrine-induced depressor responses in the dog and isoproterenol-induced relaxation of both the cat nictitating membrane and the vasculature of the isolated denervated hind limb of the dog. Pronethalol decreased the duration of the hypotensive activity of cryptenamine. The data suggest that sensitization of β adrenergic receptors, a possible increased release of epinephrine from the adrenal medulla, and stimulation of carotid reflex mechanism contribute to the over-all hypotension induced by cryptenamine.

THE DETERRENT to the wider use of the veratrum alkaloids in therapy is the narrow range between their therapeutic and emetic doses. Cryptenamine, an alkaloidal preparation prepared from *Veratrum viride* by a nonaqueous benzene triethylamine extraction procedure, has been reported to have a ratio of emetic to effective hypotensive dose superior to that of other veratrum preparations (1, 2). Finnerty (2) reported that in humans the divergence between the hypotensive and emetic doses of cryptenamine was apparent on intravenous administration. McCall and his colleagues (3) studied the effects of cryptenamine on cerebral circulation and cerebral oxygen consumption in patients with toxemia of pregnancy and reported that cryptenamine induced fewer side effects than other veratrum preparations. The satisfactory ambulatory treatment of hypertension has been reported (4). Abreu *et al.* (5), however, failed to demonstrate any superiority of cryptenamine over protoveratrine A in comparing ratio of emetic to hypotensive dose in dogs.

The pharmacological advantages of cryptenamine have been attributed to the presence of amorphous hypotensive ester alkaloids reportedly lost by hydrolysis during alternative ammonia benzene extraction procedures (1). The present investigation was mainly concerned with the study of the hypotensive activity of cryptenamine and possible mechanism of action.

METHODS

Effects of Cryptenamine on Blood Pressure and Heart Rate of Anesthetized Dogs.—Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 35 mg./Kg., i.v., and the blood pressure recorded from a cannulated femoral artery *via* a Statham pressure transducer onto a Grass polygraph. Heart rate was computed from blood pressure recordings. The intensity and duration of the hypotensive effects of control doses of cryptenamine, 5 mcg./Kg., were determined for each preparation. After arterial pressure returned to normal baseline, the animals were treated with one of the following: atropine sulfate, 1 mg./Kg.; *N,N* - di - isopropyl - *N'* - isoamyl - *N'* - diethylaminoethylurea (P-286), 10 mg./Kg.; bretylium tosylate, 10 mg./Kg.; or pronethalol, 10 mg./Kg.; and 5 mcg./Kg. of cryptenamine repeated within 10 min. All compounds were administered into a cannulated femoral vein. In several additional

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* Present address: Department of Pharmacology, U. S. Vitamin and Pharmaceutical Corp., Yonkers, N. Y.

experiments, the animals were debuffered by denervation of the carotid sinus-body complex and bilateral vagotomy or bilateral adrenalectomy performed.

Effects of Cryptenamine on Dogs Pretreated with Reserpine, Guanethidine, or α Methyldopa.—In order to study the role of catecholamines on the mechanism of cryptenamine activity, dogs were pretreated with one of the following: reserpine phosphate, 1 mg./Kg., i.m., 24 hr. prior to the experiment; α methyldopa, 200 mg./Kg., 6 hr. prior to the experiment; or guanethidine, 10 mg./Kg., i.p., 12 hr. prior to the experiment. The animals then were anesthetized with sodium pentobarbital administered by slow intravenous infusion. Blood pressure and heart rate were recorded, and cryptenamine, 5 mcg./Kg., was administered intravenously. Several of these experimental animals were also pretreated with atropine sulfate, 1 mg./Kg., i.v., prior to the administration of cryptenamine.

Effects of Cryptenamine on Epinephrine-Induced Depressor Responses on Blood Pressure of Dogs.—Small doses of epinephrine may produce depressor responses by stimulation of β adrenergic receptors. This property of epinephrine was utilized in the following experiment to study the effects of cryptenamine on β adrenergic receptors.

Mongrel dogs were anesthetized with sodium pentobarbital, 35 mg./Kg., i.v., and prepared for the recording of blood pressure as previously described and mean blood pressure recorded on a Grass polygraph. Four doses of *l*-epinephrine bitartrate, 0.025, 0.05, 0.10, and 0.15 mcg./Kg., were administered intravenously. Cryptenamine, 5 mcg./Kg., i.v., was then administered and the doses of epinephrine repeated 30 min. later.

Effects of Cryptenamine on β Adrenergic Receptors in the Cat Nictitating Membrane.—Effects of cryptenamine on β adrenergic receptors in the cat nictitating membrane were studied using the procedure described by Gyorgy *et al.* (6). Cats were anesthetized with sodium pentobarbital, 35 mg./Kg., i.p. A femoral artery was cannulated for recording of blood pressure as previously described. Preganglionic superior cervical sympathetic nerve was isolated and stimulated at a frequency of 6 c.p.s., 0.2 msec. duration, and 1–3 v., thereby producing a state of continuous contraction of the nictitating membrane without damage to either nerve or muscle. Under these conditions, relaxation of the membrane was elicited with the administration of 5 to 20 mcg./Kg. of isoproterenol, i.v. Cryptenamine, 5 mcg./Kg., was then administered and the same dose of isoproterenol repeated. Both the blood pressure and the activity of the nictitating membrane were recorded on a Grass polygraph.

Effects of Cryptenamine on Isoproterenol-Induced Relaxation in the Denervated Perfused Hind Limb of the Dog.—Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 35 mg./Kg., i.v., and a femoral artery was cannulated for the recording of blood pressure. One hind limb was denervated by severing the femoral and sciatic nerve trunks, and vascularly isolated by clamping the muscles with a 21-gauge stainless steel wire placed under the femoral artery and vein and ligated with a Schiffrin wire tightener. The distal segment of the femoral artery of the isolated limb was cannulated and perfused with the blood drawn from the central segment of the same artery. A sigmamotor pump was utilized to perfuse the leg at a constant rate of flow. Perfusion pressure was measured between the pump and isolated limb by means of a Statham pressure transducer. The changes in perfusion could be related to the changes in the vascular resistance, since blood flow to the limb remained relatively constant.

In order to study the effects of cryptenamine on β adrenergic receptors, four different doses of isoproterenol, 1, 2, 5, and 10 mcg., were administered intra-arterially into the limb followed by cryptenamine, 5 mcg./Kg., i.v. After a 30-min. stabilization period, the doses of isoproterenol were repeated.

Effects of Cryptenamine in the Dog Cross-Circulation Preparation.—Recipient dogs were anesthetized with sodium pentobarbital, 35 mg./Kg., i.v., and prepared for the recording of blood pressure as previously described. The neck musculature was removed utilizing electrocautery to expose the vertebral column from C-2 to C-5. A dorsal laminectomy was performed between C-3 and C-4; the vertebral venous sinuses and vertebral arteries were occluded utilizing 21-gauge stainless steel wire, as described by Bickerton and Buckley (7). Donor dog was similarly anesthetized and prepared for the recording of blood pressure. Circulation was established between the right common carotid artery of the donor and the head of the recipient *via* the recipient's two common carotid arteries and from the two jugular veins of the recipient's head to the right jugular vein of the donor animal. This resulted in a neurally intact, vascularly isolated recipient's head preparation. Circulatory leakage between the recipient's head and the trunk was determined utilizing ^{131}I (radio-iodinated serum albumin) administered into the recipient's carotid inflow. In several of these preparations, the carotid sinus-body complex of the recipient was denervated. Cryptenamine, 5 mcg./Kg., was administered into the arterial inflow of the recipient's head.

Effects of Intraventricular Administration of Cryptenamine on Blood Pressure of Cats.—The method of administration of compounds into the cerebrolateral ventricle, as described by Feldberg (8) and Bhattacharya and Feldberg (9), was utilized in these experiments. Cats were anesthetized with sodium pentobarbital, 35 mg./Kg., i.p., and prepared for the recording of blood pressure on an Offner dynograph as described earlier. A 22-gauge unbeveled needle, 34 mm. in length, employed as a cannula, was stereotaxically implanted in the left cerebrolateral ventricle and secured firmly to the skull with dental cement. The cisterna magna was exposed and the dura carefully cut allowing the cerebrospinal fluid to escape. Then, the lateral ventricle was perfused with artificial cerebrospinal fluid (NaCl, 8.1 Gm.; KCl, 0.25 Gm.; $CaCl_2$, 0.14 Gm.; $MgCl_2$, 0.11 Gm.; NaH_2PO_4, 0.081 Gm.; $NaHCO_3$, 0.07 Gm.; urea, 0.13 Gm., and dextrose, 0.61 Gm.; glass distilled water, 1000 ml.) maintained at 38°. The rate of perfusion was kept constant at 0.1 ml./min., utilizing a Phipps Bird constant-infusion pump.

Lateral ventricles were perfused 60 to 90 min. prior to the administration of cryptenamine. Doses of cryptenamine ranging from 1 to 10 mcg. were administered into the ventricles utilizing a Baltimore microinjection unit.

TABLE I.—EFFECTS OF CRYPTENAMINE, 5 mcg./Kg., i.v., ON BLOOD PRESSURE AND HEART RATE OF DOGS PRETREATED WITH SEVERAL PHARMACOLOGICAL AGENTS

Pretreatment	Dose, mg./Kg.	Animals, No.	Prior to Pretreatment: Mean % Decrease in Blood Pressure	Prior to Pretreatment: Mean % Decrease in Heart Rate	Prior to Pretreatment: Mean Duration, min.	Following Pretreatment: Mean % Decrease in Blood Pressure	Following Pretreatment: Mean % Decrease in Heart Rate	Following Pretreatment: Mean Duration, min.
Atropine	1	4	49.4	35.02	132.0	35.1	24.4	113.8+
Adrenalectomy	...	3	49.3	32.7	83.3	29.5	21.1	59.0
Adrenalectomy + atropine	... 1	3	49.8	29.3	115.0	37.2	3.7	85.0
Adrenalectomy + atropine	... 1	3	48.0	43.5	134.3	17.9	7.8	2.5
P-286	10	3	43.2	37.5	156.7	15.5	23.0	36.3
P-286 + atropine	10 1	3	44.3	29.2	186.7	19.0	5.0	4.3
Bretylium	10	3	52.2	32.0	87.3	28.7	10.1	82.7
Bretylium + atropine	10 1	3	52.5	28.6	60.3	0	0	...
Pronethalol	10	4	44.0	37.7	119.5	28.7	17.9	85.2
Pronethalol + atropine	10 1	4	50.5	37.9	131.2	39.2	1.3	85.2
Carotid sinus denervation + vagotomy		3	54.3	51.4	105.0	0	0	...
Reserpine	1	3				31.2	30.8	34.0
Reserpine + atropine	1 1	4				5.1	0	5.0
α Methyldopa	200	3				34.9	35.8	44.0
α Methyldopa + atropine	200 1	4				10.4	2.5	1.9
Guanethidine + atropine	10 1	4				41.9	0	41.2+

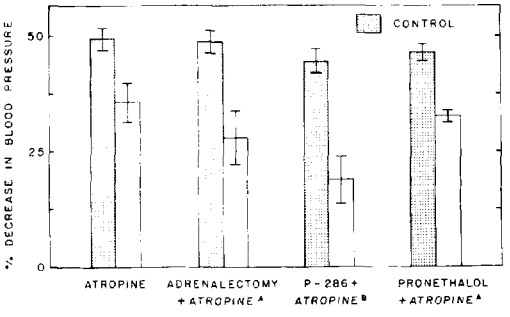

Fig. 1.—Effect of cryptenamine (5 mcg./Kg., i.v.) on blood pressure of dogs pretreated with certain pharmacological agents. Key: A, shorter duration compared with control responses; B, duration less than 10 min.

RESULTS

Effects of Cryptenamine on Blood Pressure and Heart Rate of Anesthetized Dogs.—Single doses of cryptenamine, 5 mcg./Kg., produced marked bradycardia and hypotension, persisting for 2 to 3 hr. A return of the bilateral carotid occlusion response was taken as an index for the recovery of the animals from the effects of cryptenamine. Tachyphylaxis was not observed when the second dose of cryptenamine was administered after return of this reflex.

Pretreatment of the animals with atropine sulfate partially blocked the bradycardia and the depressor effect. When cryptenamine was administered after adrenalectomy or pretreatment with P-286 or bretylium, the onset of action was delayed and only a moderate decrease in blood pressure occurred for a relatively short period of time. Pretreatment with either bretylium or P-286 produced complete blockade of the effects of cryptenamine in atropinized dogs. The cryptenamine depressor effect was blocked in three of six atropine-treated adrenalectomized animals, and the duration of activity was significantly reduced in the other animals. Pretreatment with pronethalol or pronethalol plus atropine sulfate decreased the duration of the hypotensive effects of cryptenamine. Bilateral vagotomy or bilateral denervation of the carotid sinus-body complex by itself was not sufficient to completely block the effects of cryptenamine; however, the effects were abolished by complete debuffering (Table I, Fig. 1).

Effects of Cryptenamine on Dogs Pretreated with Reserpine, Guanethidine, or α Methyldopa.—Pretreatment of the animals with either reserpine or α methyldopa significantly reduced the intensity and duration of the hypotensive activity of cryptenamine. Pretreatment with the above compounds followed by atropinization completely eliminated the depressor activity of cryptenamine. Guanethidine plus atropine abolished the effects of cryptenamine on heart rate but failed to reduce markedly the hypotensive activity of cryptenamine (Table I).

Effects of Cryptenamine on Epinephrine-Induced Depressor Responses on Blood Pressure of Dogs.—Depressor responses of epinephrine increased with the doses in the dosage range utilized. After cryptenamine, the depressor responses produced by each dose of epinephrine were potentiated suggesting a sensitization of β adrenergic receptors by cryptenamine (Table II).

Effects of Cryptenamine on β Adrenergic Receptors in the Cat Nictitating Membrane Preparation.—Isoproterenol induced a transient relaxation

of the electrically contracted nictitating membrane, and administration of cryptenamine did not alter the state of the contraction of the membrane. However, following cryptenamine, the administration of isoproterenol produced a biphasic response consisting of an initial relaxation of the membrane approximately equal to the control responses followed by a secondary relaxing effect of greater intensity and duration of action, suggesting a potentiation of the isoproterenol response on the nictitating membrane. When isoproterenol was administered following a return of the cat's blood pressure to precryptenamine levels, the effects on the nictitating membrane were similar to control responses both in intensity and duration (Table III).

Effects of Cryptenamine on Isoproterenol-Induced Relaxation in the Denervated Perfused Hind Limb of the Dog.—Administration of isoproterenol intra-arterially into the denervated perfused hind limb produced a decrease in perfusion pressure. The decrease in vascular resistance as indicated by a decrease in perfusion pressure was proportional with the dose of isoproterenol in that there was a linear relationship between the decrease in perfusion pressure and log dose of the β adrenergic stimulant. Doses of isoproterenol were repeated 20 to 30 min. after the administration of cryptenamine, and the decrease in perfusion pressure originally produced by each dose was significantly potentiated, approximately 25 to 30%. On graphical representation, the log-dose response relationship shifted to the left indicating the potentiation facilitated by cryptenamine on isoproterenol-induced relaxation of the vasculature of the limb (Table IV, Fig. 2).

Effects of Cryptenamine in Dog Cross-Circulation Preparation.—The administration of cryptenamine into the carotid inflow of the recipient's head produced a significant decrease in blood pressure in the recipient's trunk followed by a decrease in the donor blood pressure (N = 4). When the animals were debuffered by denervation of the carotid sinus-body complex, administration of cryptenamine into the head produced a pressor response in the body of the recipient followed by a depressor response in the donor (Table V).

Effects of Intraventricular Administration of Cryptenamine on Blood Pressure of Cats.—Doses ranging from 1 to 10 mcg. of cryptenamine were administered into the perfused lateral ventricle of cats. The lower doses (1–2 mcg.) produced a pressor response, and higher doses (3–10 mcg.) had no effect on blood pressure of the cat. Depressor responses were not observed following the administration of cryptenamine (Table VI).

TABLE II.—EFFECTS OF CRYPTENAMINE, 5 MCG./KG., I.V., ON EPINEPHRINE-INDUCED DEPRESSOR RESPONSES ON BLOOD PRESSURE OF ANESTHETIZED DOGS

	Before Cryptenamine—Decrease in Mean Blood Pressure to Epinephrine, mm. Hg				After Cryptenamine—Decrease in Mean Blood Pressure to Epinephrine, mm. Hg				Change, %			
Wt., Kg., Sex	0.025	0.05	0.10	0.15	0.025	0.05	0.10	0.15	0.025	0.05	0.10	0.15
	mcg./Kg.				mcg./Kg.				mcg./Kg.			
13.7 M	5	9	12.5	...	10	15	18	...	100	66.6	44.0	...
15.4 F	5	5	10.0	15.0	15	10	20	25	200	100.0	100.0	66.6
13.4 F	5	15	30.0	25.0	20	20	35	35	300	33.3	16.6	40.0
14.2 M	0	5	7.5	12.5	8	10	13	24	α	100.0	73.3	92.0
Mean	3.75	8.5	15.0	17.5	13.25[a]	13.75[a]	21.5[a]	28.0[a]				
± S. E.	±1.25	±2.36	±5.11	±3.83	±2.69	±2.40	±4.73	±3.52				

[a] Significantly different from pretreatment response ($P < 0.05$) when t values calculated by direct difference method (15).

TABLE III.—EFFECTS OF CRYPTENAMINE, 5 MCG./KG., ON ISOPROTERENOL-INDUCED RELAXATION OF THE CAT NICTITATING MEMBRANE

			Before Cryptenamine				After Cryptenamine			
Sex	Wt., Kg.	Isoproterenol Dose, mg./Kg.	Decrease in Blood Pressure, %	Duration, min.	Relaxation of N. M., mm.	Duration, min.	Decrease in Blood Pressure, %	Duration min.	Relaxation of N. M., mm.	Duration, min.
F	3.6	20	58	6.0	18	3.0	61	30.0	22	11.5
M	3.2	10	65	11.0	13	2.0	71	10.0	18	10.0+
F	2.8	10	51	4.0	7	4.6	52	4.0	8	10.0+
M	2.6	10	30	3.6	11	3.3	46	4.3	18	12.0+

TABLE IV.—EFFECTS OF CRYPTENAMINE, 5 MCG./KG., I.V., ON ISOPROTERENOL-INDUCED RELAXATION IN DENERVATED PERFUSED HIND LIMB OF THE DOG

Dose of Isoproterenol, mcg.	Animals, No.	Mean Decrease in Perfusion Pressure, mm. Hg ± S. E.—Before Cryptenamine	After Cryptenamine	Probability (P)[a]
1	6	34.8 ± 3.69	45.2 ± 5.62	0.01
2	6	41.0 ± 3.91	55.25 ± 5.53	0.01
5	6	48.0 ± 4.92	62.8 ± 4.21	0.01
10	6	52.5 ± 5.12	67.5 ± 5.32	0.01

[a] P was calculated by direct difference method (15).

DISCUSSION

Veratrum alkaloids have been reported to sensitize certain afferent receptors in left ventricles sending impulses *via* the vagus to the central nervous system resulting in hypotension and bradycardia (10). Cryptenamine appears to resemble other veratrum derivatives in this respect. It was necessary to perform bilateral vagotomy as well as bilateral denervation of the carotid sinus-body complex to abolish the effects of cryptenamine on blood pressure and heart rate, suggesting that cryptenamine induced reflex hypotension and bradycardia by affecting afferent receptors at the carotid sinus-body area and in the myocardium. Central hypotensive effects of cryptenamine were not demonstrated either in cross-circulation preparations or in perfused lateral ventricle preparations of the cat. The depressor effects observed in the recipient's trunk following the administration of cryptenamine into the arterial inflow of the head in the dog cross-circulation preparations were most likely due to the effects of cryptenamine on baro- or chemo-receptors in the carotid sinus-body complex. These effects were abolished in the debuffered preparations, and pressor effects resulted in the recipient's trunk.

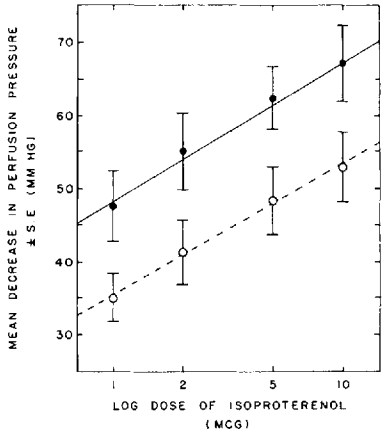

Fig. 2.—Effect of cryptenamine on isoproterenol responses in denervated perfused hind limb of dog. Crytenamine, 5 mcg./Kg. Key: ○, before; ●, after.

The efferent pathways in this reflex are still open to question. None of the pharmacological agents (atropine, pronethalol, bretylium, and P-286) when administered alone, could block the cryptenamine effects in dogs even though they all produced marked alterations in the responses, suggesting the complexity of various mechanisms involved in this reflex. The efferent parasympathetic pathways were blocked by atropinization in order to study the nature of other efferent pathways.

P-286 has been reported to block catecholamine release from the adrenal medulla induced by splanchnic stimulation (11). Pretreatment of the animals with P-286 and atropine sulfate completely abolished the effects of cryptenamine on blood pressure and heart rate. If P-286 is as specific in blocking the adrenal glands as has been reported, a combination of adrenalectomy and atropinization would be expected to produce similar effects. The data obtained in the current study do not totally disagree with this possibility, in that adrenalectomy produced marked inhibition of cryptenamine activity in atropinized animals. Since the adrenal medulla contains large concentrations of catecholamines especially epinephrine, it is quite probable that cryptenamine may reflexly release epinephrine from the adrenal medulla. The possible role of catecholamines in the hypotensive mechanisms of cryptenamine was further confirmed using animals

TABLE V.—EFFECTS OF CRYPTENAMINE, 5 mcg./Kg., i.v., ON BLOOD PRESSURE OF RECIPIENT AND DONOR DOGS IN CROSS-CIRCULATION PREPARATIONS

Expt.	Control Blood Pressure, mm. Hg		Change in Blood Pressure, %				Change in Perfusion Pressure, %	Duration, min.
	Donor	Recipient	Donor	Duration, min.	Recipient	Duration, min.		
1	125/100	120/75	−46.2	75+	−31.1	75+	−12.5	75+
2	125/95	120/95	−50.4	45+	−24.2	45+	−30.3	30
3	175/115	105/75	−45.9	85+	−47.0	47	−30.0	40
4	140/110	150/120	−45.8	55	−63.0	55+	−34.7	20
5[a]	200/135	100/60	−69.4	45	+36.9	55+	−12.5	55
6[a]	160/120	100/55	−52.6	70+	+61.4	70+	−33.3	35
7[a]	175/110	110/60	−53.0	75+	+49.3	75+	−25.0	42
8[b]	120/95	90/35	−30.0	35+	0.0	...	−10.7	35+

[a] Recipient dogs were debuffered. [b] Recipient pretreated with bretylium tosylate (10 mg./Kg. *via* femoral vein).

TABLE VI.—EFFECTS OF INTRAVENTRICULAR ADMINISTRATION OF CRYPTENAMINE ON BLOOD PRESSURE OF CATS

Sex	Wt., Kg.	Dose, mcg.	Control Blood Pressure, mm. Hg	Change, %	Duration, min.
F	2.6	1	110/60	+33.7	160
M	2.3	2	190/110	+ 7.8	150
M	2.3	3	190/110	0.0	...
M	3.4	5	190/125	0.0	...
M	3.4	10	190/125	0.0	...

pretreated with reserpine or α methyldopa. Depletion of catecholamines with reserpine or α methyldopa (12) facilitated blockade of the effects of cryptenamine in atropinized animals, whereas guanethidine which does not induce depletion of catecholamines from the adrenal medulla (13) could not block the cryptenamine effects in animals pretreated with atropine sulfate. Therefore, it appears that nonparasympathetic efferent pathways involved in the reflex hypotension produced by cryptenamine may exert their action through the release of catecholamines from the adrenal medulla.

Since the effects of epinephrine are predominantly on α receptors (14), a sensitization of β receptors could mask any pressor effect that might result from epinephrine. The data obtained indicate a sensitization of β adrenergic receptors following the administration of cryptenamine.

It is apparent that there are many mechanisms in the complex phenomena of cryptenamine-induced hypotension. The results of this investigation which included the ability of P-286, reserpine, α methyldopa, and adrenalectomy to inhibit the effects of cryptenamine suggest a role of catecholamines and the adrenal medulla. The reduction of the cryptenamine effects by pronethalol, potentiation of the isoproterenol effects in the cat nictitating membrane and isolated perfused hind limb of the dog, and potentiation of the epinephrine-induced depressor responses in the dog indicate a role of the β adrenergic receptors and suggest that cryptenamine sensitizes the β adrenergic receptors to circulating epinephrine.

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Formation of Acetylcodeine from Aspirin and Codeine

By A. L. JACOBS, A. E. DILATUSH, S. WEINSTEIN, and J. J. WINDHEUSER

A reaction is discussed which leads to the formation of acetylcodeine from aspirin and codeine. It is noted that the generally published methods of analysis will not differentiate between the two alkaloids. A method of separating and assaying the individual compounds is described. Furthermore, the dependence of the interaction on water is discussed.

Although an abundance of products are marketed which contain combinations of aspirin, phenacetin, caffeine, and codeine, little has been published as to the stability and reactivity of these systems. Studies (1, 2) have been conducted on the stability of aspirin *per se*, but little attention has been given to its effect on other compounds.

During the development of a capsule product containing aspirin, phenacetin, caffeine, itobarbital, and codeine phosphate,[1] thin-layer chromatography indicated the presence of an unknown product in some samples after aging. The present communication deals with an investigation of this reaction and indicates that under certain conditions acetylcodeine forms. The acetylcodeine which results from this interaction of aspirin and codeine cannot be detected by the normal analytical methods employed for the determination of codeine. A partition column separation technique of codeine from acetylcodeine is described.

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[1] Fiorinal plus Codeine Capsules, Sandoz Pharmaceuticals, Hanover, N. J.

RESULTS AND DISCUSSION

Isolation and Identification of the Reaction Product.—Based upon the possible reactants, it was speculated that an interaction might occur between aspirin and codeine (Scheme I).

Although the mechanism of the reaction has not been investigated, it might proceed by a classical transesterification or might be facilitated by an